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β-GALACTOSIDASE GENE AS SELECTION MARKER FOR EXPRESSION VECTORS OF *BOMBYX MORI* NUCLEAR POLYHEDROSIS VIRUS

NIEN-TAI HU[1], PEIH-RUR LIN[2] and ROGER F. HOU[2]

Agricultural Biotechnology Laboratories[1], and Department of Entomology[2], National Chung Hsing University, Taichung, Taiwan 40227, Republic of China

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Nien-Tai Hu, Peih-Rur Lin and Roger F. Hou (1991) β-Galactosidae gene as selection marker for expression vectors of *Bombyx mori* nuclear polyhedrosis virus. *Bull. Inst. Zool., Academia Sinica* **30**(2): 87-98. Transplacement plasmids utilizing the polyhedrin gene promoter of *Bombyx mori* nuclear polyhedrosis virus (BmNPV) have been constructed in this laboratory. Production of hepatitis B virus surface antigen (HBsAg) from the *Bombyx mori* cells and the silkworms infected with the recombinant virus has also been demonstrated. While the absence of occulsion bodies (Occ^-) was taken as the selection marker, it was somewhat difficult to identify the recombinant viruses without experienced eyes. Thus, the selection had to rely upon the time-comsuming, but less efficient, hybridization method. In order to improve the selection method, the *lacZ* gene of *Escherichia coli* was introduced into the BmNPV transplacement plasmids. A set of six, each with two polyhedrin gene promoters, were constructed. One of the two promoters was used to promote the transcription of the *lacZ* gene, while the other was left with the cloning site at the appropriate location for the expression of a foreign gene. The gene encoding the major S protein of hepatitis B virus was cloned into such transplacement plasmids. Production of the recombinant viruses containing the HBsAg gene was accomplished by cotransfection of BmN cells with the recombinant plasmid and BmNPV DNA. Blue plaques were observed on plates containing the indicator for β-galactosidase, X-gal. No other effort was needed to distinguish them from the surrounding white plaques produced by the wild type BmNPV. Through four or five rounds of blue plaque picking, purified recombinant virus without any polyhedra was obtained from the blue plaque. The *lacZ* gene expression has certainly provided a convenient alternative for the selection of the recombinants.

Key words: Baculovirus expression vectors, β-galactosidase gene expression, Blue plaques, HBsAg expression, BmNPV.

Baculovirus expression vectors have been widely used in synthesizing heterologous proteins in insect cells (Luckow and Summers, 1988; Maeda, 1989a). Transplacement plasmids which contained the polyherdin gene promoter and the flanking sequences served as an intermediate in the construction of recombinant viruses for the expression of foreign genes in insect cells. Cotransfection of insect cells with the wild type Baculovirus DNA and the transplacement plasmid containing the foreign gene allowed the foreign

gene inserted downstream of the polyhedrin gene promoter to replace the wild type polyhedrin gene. Isolation of recombinant viruses from among 100- to 1,000-fold more wild type viruses has been relying upon the absence of occlusion bodies (Occ^-) in cells infected by the recombinant viruses (Luckow and Summers, 1988; Miller, 1988). However, wild type BmNPV formed precipitate-like plaques on BmN cells (Hung and Hu, unpublished observation). Being Occ^-, the recombinant virus did not form the precipitate-like plaques, nor clear plaques. This made the screening for Occ^- plaques not only time-consuming but also inaccurate. The problem has been improved by cloning a subline of BmN cells which formed clear plaques while infected by Occ^- virus (Maeda, 1989b). In our case, we decided to introduce an additional screening marker into our BmNPV transplacement plasmids. The *lacZ* gene of *Escherichia coli* was chosen, because an AcNPV polyhedrin/β-galactosidase fusion gene was shown to give rise to blue plaques in *Spodoptera frugiperda* cells (Pennock *et al.*, 1984). A similar fusion gene was introduced into the BmNPV transplacement plasmids which were constructed by Hung (1989). Recombinant viruses containing the gene encoding the surface antigen of hepatitis B virus (HBsAg) was plaque purified using the blue color produced from the degradation of X-gal by β-galactosidase as a marker. The production of HBsAg in BmN cells was compared to that produced by a control recombinant virus which contained an identical construction of HBsAg gene but lacked the second polyhedrin gene promoter.

MATERIALS AND METHODS

Plasmids, virus and cell line

The plasmid pAV5/β-gal was a kind gift of Dr. M. Fraser. The plasmid pTWS1 containing the gene encoding the major S of hepatitis B virus was made available by Dr. K. B. Choo and the plasmid pHBVMM containing the gene encoding the middle S gene with a G to A change on the third base upstream of ATG and a C to G change on the fourth base from the A of the initiation codon came from Dr. S. J. Lo. All other plasmids used in the constructions were obtained from our previous work. The recombinant virus BmHBs324P was constructed by Hung (1989). The BmN cell line was obtained from Ms. M. Funakoshi.

Reagents, buffers and enzymes

The low melting point agarose (SeaPlaque^R agarose) used in the plaque assay was purchased from FMC. The transfection reagent (DOTMA) was purchased from Boehringer Mannheim. An EverNew Monoclonal HBsAg EIA kit was used in the ELISA assay. The other buffers used in the cell culture, cotransfection and the plaque assay were according to Summers and Smith (1987). Enzymes used in plasmid constructions were purchased from Boehringer Mannheim.

Isolation of polyhedra

Fifth instar larvae of silkworms were infected with BmNPV by feeding them mulberry leaves covered with virus suspension. On the fifth day post-infection, the moribund larvae were collected and stored at −20°C for polyhedral isolation. The larvae suspended in water were broken apart in a blender. The filtrate passing through the cheesecloth was centrifuged at 1,500×g for 10 min. The collected pellet, resuspended in a solution composed of distilled water, *n*-butanol and *n*-hexane (at ratio of 10:4:1), was blended further in polytron and centrifuged as before. The pellet resuspended in 0.05 M Tris-HCl, pH 8.0 was layered over a 30%-80% sucrose gradient at 8,100×g for 30 min. A turbid

band near the bottom of the gradient was collected and washed three times with 0.05 M Tris-HCl, pH 8.0, followed by centrifugation at 1,500×g for 10 min. After the last centrifugation, the white pellet containing the occluded virus was lyopholized for storage or resuspended in 0 05 M Tris-HCl, pH 8.0 for the purification of BmNPV DNA (Lin, 1990).

Purification of BmNPV DNA

BmNPV DNA was purified from 500 mg polyhedra following the procedures of Corsaro and Fraser (1988). BmNPV DNA was released from the viral particles by treatment with quanidinium isothiocyanate, and then purified through two CsCl step gradients in the presence of ethidium bromide. The supercoiled DNA, recovered from the second CsCl gradient, was treated with isoamyl alcohol to remove ethidium bromide followed by dialysis against 0.1×SSC (Maniatis *et al.*, 1982).

Construction of *lacZ*$^+$ transplacement plasmids

All recombinant DNA techniques used were according to Maniatis *et al.* (1982). The chimeric gene, composed of the *Autographa californica* nuclear polyhedrosis virus (AcNPV) polyhedrin gene promoter and the *lacZ* gene of *Escherichia coli*, was recovered from pAV5/β-gal as a *Sma*I-*Eco*RV fragment. For the construction of the fusion plasmids, pBmT538 (+38), pBmT638 (+38), pBmT539 (+39) and pBmT639 (+39), the chimeric gene was introduced into pPD413 (+38) and pPD436 (+39) (Hung, 1989), respectively, at the *Hpa*I site upstream of the promoter. The number in parentheses designates the number of nucleotides of the coding sequence of BmNPV polyhedrin gene present upstream of the cloning site. For each BmNPV polyhedrin gene promoter, two clones were obtained, one with the chimeric gene transcribed in the opposite direction to that of the BmNPV polyhedrin gene (pPD538 and pPD539) and the other with the same direction of transcription (pPD638 and pPD639). In order to complete the construction, a *Bam*HI-*Eco*RI fragment from pXBE, which contained sequences downstream of the BmNPV polyhedrin gene, was inserted into each of the pPD plasmids (Fig. 1).

A different strategy was used in the construction of the nonfusion plasmids, pBmT524 (−18) and pBmT624 (−18). The number in parentheses designates, in this case, the number of nucleotides deleted upstream of the initiation codon of the BmNPV polyhedrin gene. In order to facilitate the construction, the downstream sequence was introduced into pPD324 (−18) before the insertion of the chimeric gene (Fig. 2). Again, the chimeric gene in pBmT524 was transcribed in the opposite direction to that of the BmNPV polyhedrin gene, whereas the two promoters in pBmT624 were in the same direction of transcription.

Construction of plasmids containing HBsAg genes

The nonfusion transplacement plasmids pBmT524 and pBmT624 were chosen for this construction. The gene encoding the major S of HBV was recovered from pTWS1 as a *Bam*HI fragment. The *Bam*HI-generated ends were filled with dNTP using Klenow enzyme and ligated into the *Xba*I site of the plasmids, with the ends made blunt as before. The gene encoding the middle S was recovered from pHBVMMS as a *Sal*I-*Sma*I fragment and cloned into pBmT524. All constructions were confirmed by various combinations of restriction enzyme digestions.

Cotransfection and plaque purification

Construction of recombinant virus with foreign genes replacing the polyhedrin gene of the wild type BmNPV was made possible *via* introduction of

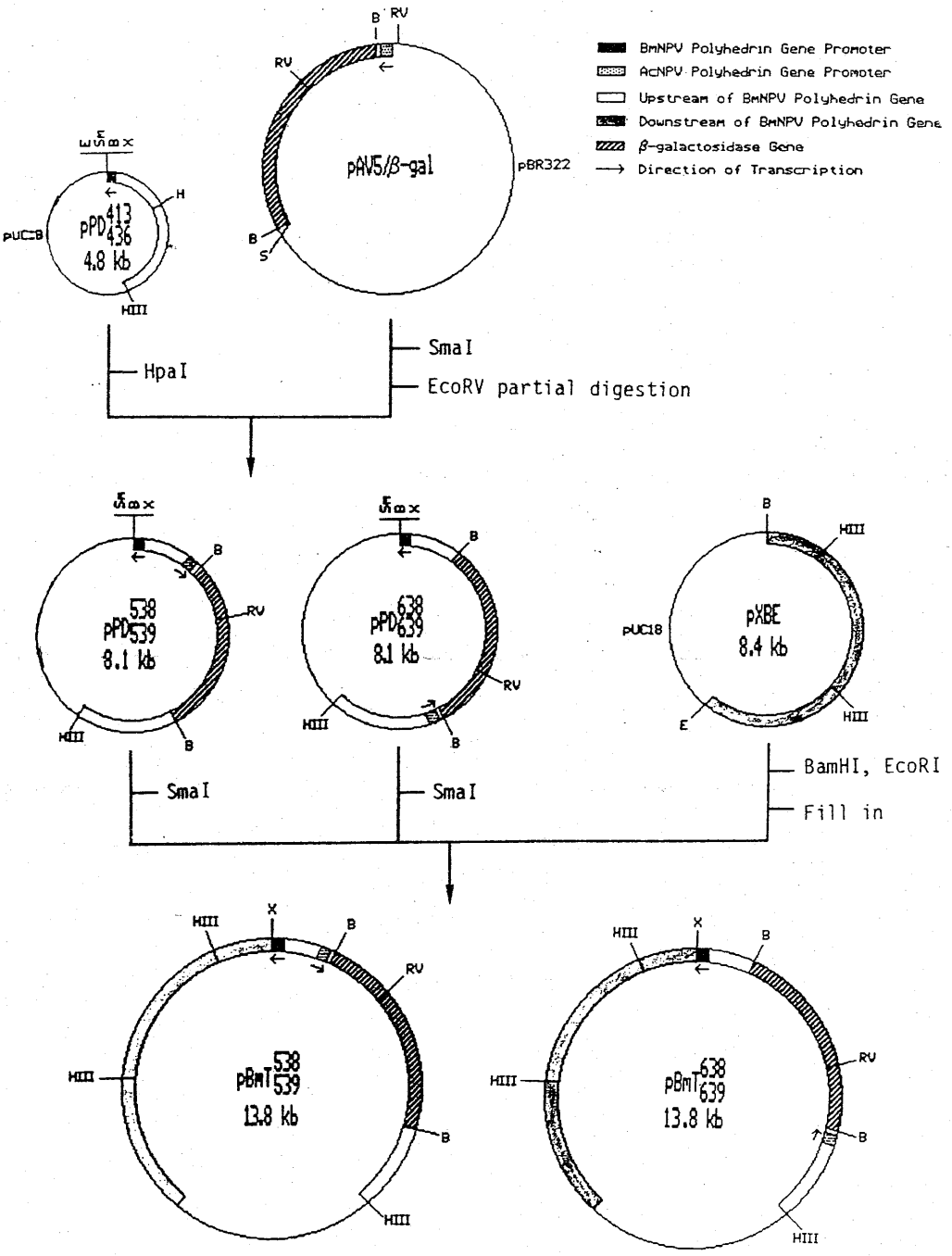


Fig. 1. Construction of BmNPV transplacement fusion plasmids: pBmT538, pBmT539, pBmT638, and pBmT639. Abbreviations are as follows: B, *Bam*HI; E, *Eco*RI; H, *Hpa*I; HIII, *Hin*dIII; RV, *Eco*RV; Sm, *Sma*I and X, *Xba*I.

both wild type BmNPV DNA and the recombinant plasmid containing the foreign genes. Calcium phosphate precipitation (Summers and Smith, 1987) or a transfection-agent (DOTMA)-mediated method (Felgner *et al.*, 1987) was followed in the cotransfection of BmN cells with BmNPV DNA and the recombinant DNA

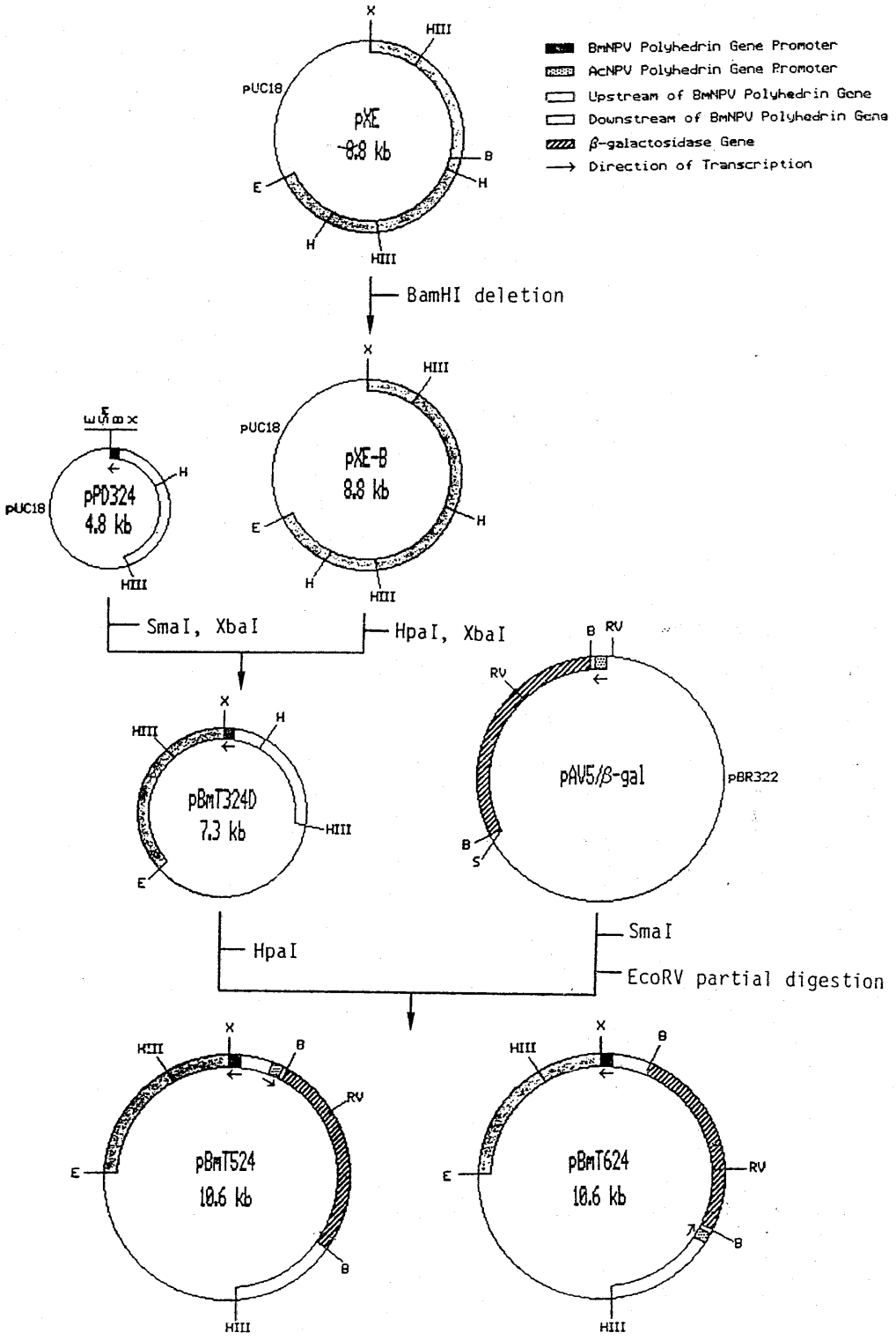


Fig. 2. Construction of BmNPV transplacement nonfusion plasmids: pBmT524 and pBmT624. Abbreviations are as in Fig. 1.

containing HBsAg gene. The plaque assay was according to Summer and Smith (1987) with slight modifications. In the overlay agarose, 5-bromo-4-chloryl-3-indolyl-β-D-galactopyranoide (X-gal) was included at 150 μg/ml. Blue plaques appeared after 3-5 days of incubation at 28°C. Contaminated with wild type BmNPV, the recombinant virus was purified through four to five rounds of plaque picking, dilutions and plaque assay.

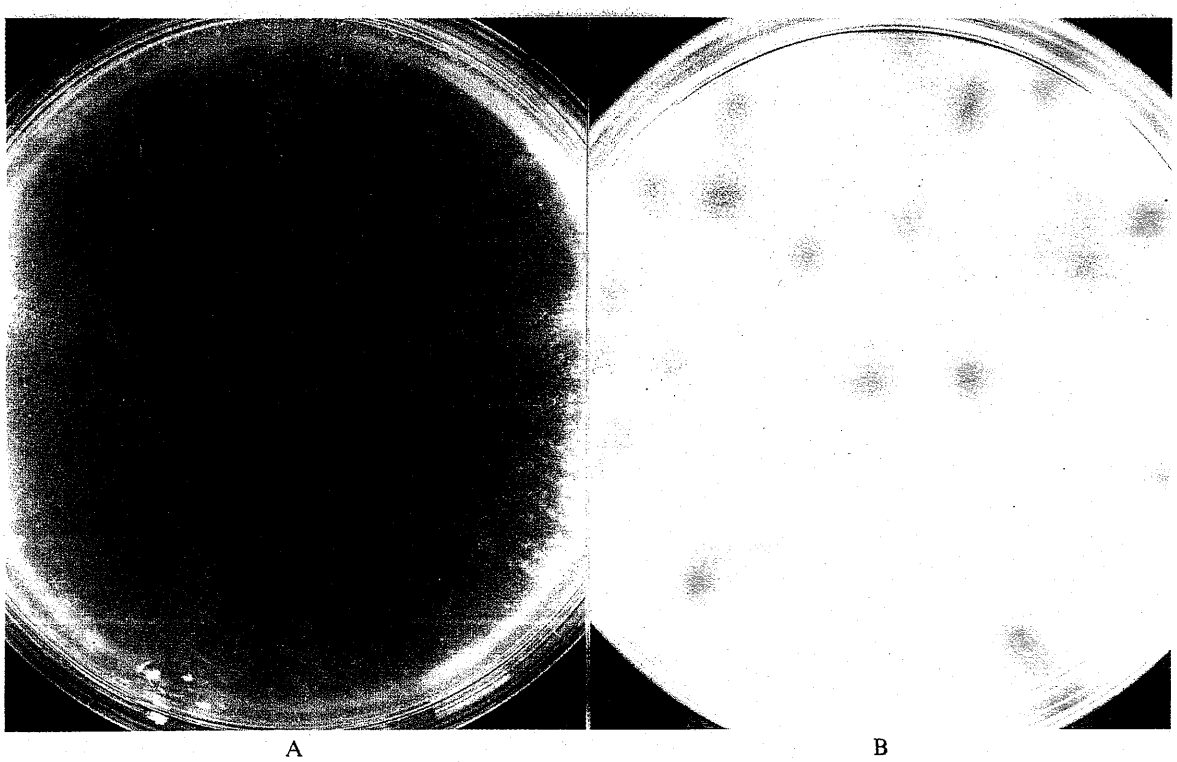
ELISA assay of HBsAg

BmN cells at 1.2×10^6 per dish (60×15 mm) were infected with viruses at an MOI of 1. Both culture medium and cell extract were collected on the first to the fourth day post-infection. The cell extract was prepared by sonication of PBS-washed cells, followed by centrifugation at 10,000 rpm for 20 min. The supernatant collected from the centrifugation was taken as the cell extract. The amount of HBsAg in each sample was determined by ELISA assay using the EverNew Monoclonal HBsAg EIA kit. Samples with absorbancy at 490 nm higher than 1 were diluted serially to the point that the absorbance was within the liner range (0.2-1.0 OD_{490}) of the standard curve.

RESULTS

Expression of *lacZ* gene of *E. coli* in BmN cells

Cotransfection of BmN cells with pAV5/β/gal and BmNPV DNA gave rise to a blue plaque on plates supplemented with X-gal. This result suggested that the *lacZ* gene under the control of the polyhedrin gene promotor of AcNPV can be expressed in BmN cells. The cassette



A B

Fig. 3. Plaque assay with X-gal in the agarose overlay, photographed with light above the plate on two sides (A) and with light underneath plate (B). In (A) "rec" indicates the blue plaque formed by recombinant virus, "wt" points to the white plaque formed by the wild type virus.

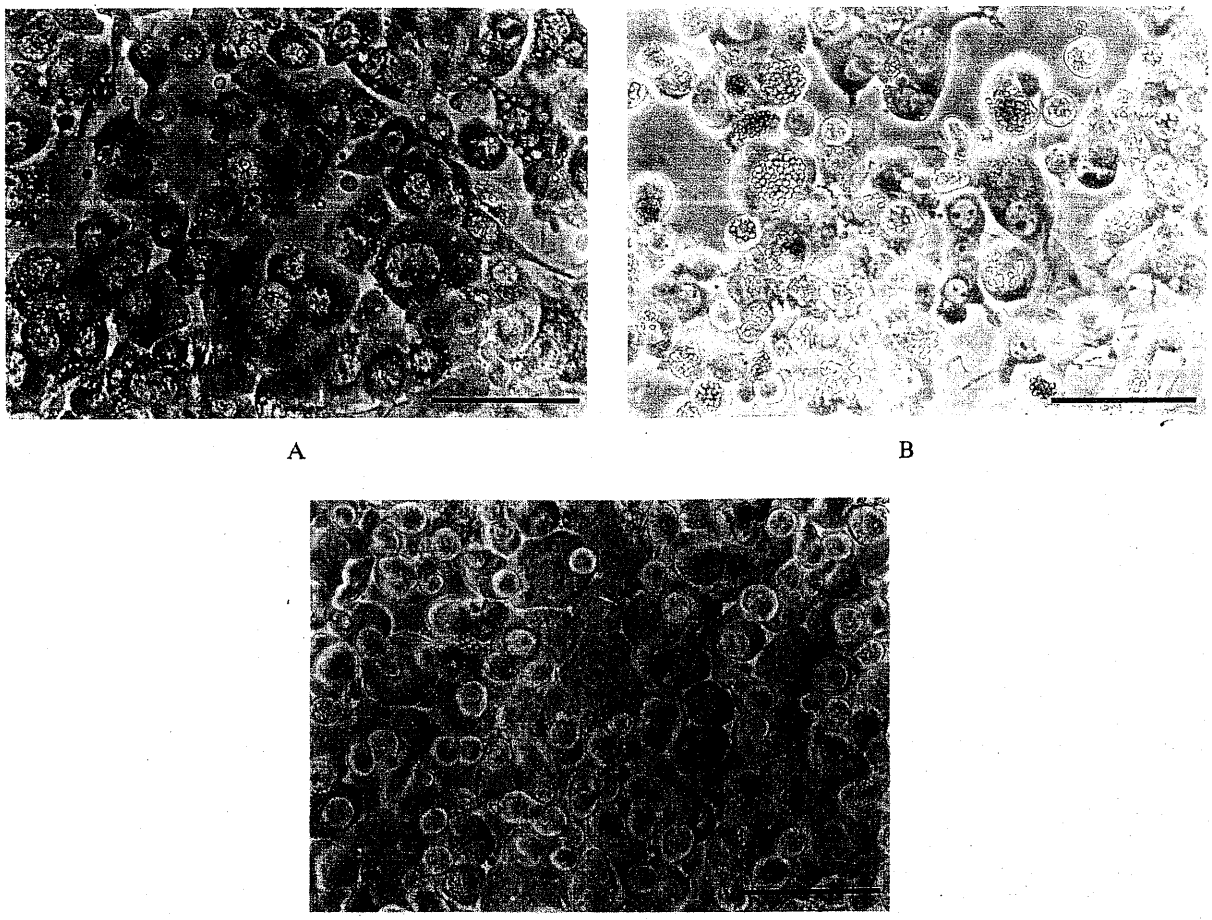


Fig. 4. BmNPV-infected BmN cells in blue plaque observed under the inverted microscope. (A) infected with recombinant virus containing *lacZ* gene on the 4th day post-infection; (B) infected with type BmNPV; (C) uninfected cells. (Bar=100 μm)

containing the AcNPV polyhedrin gene promoter and the *lacZ* gene from pAV5/ β-gal was thus introduced into the BmNPV transplacement plasmids constructed previously in this laboratory (Hung, 1989). The original BmNPV polyhedrin gene promoter was left available for expressing the foreign gene. Cotransfection of BmN cells with the newly constructed transplacement vectors and BmNPV DNA again yielded blue plaques on X-gal plats (Fig. 3). Three to five days post-infection, the cells infected by the LacZ$^+$ recombinant virus locked blue under the inverted microscope with no polyhedron in the nucleus (Fig. 4A). In contrast, those infected by the wild type virus looked colorless with polyhedra-filled nucleus (Fig. 4B) and uninfected cells had neither polyhedra nor color (Fig. 4C).

Purification of recombinant virus containing the HBsAg gene from blue plaques on X-gal plate

The middle S and the major S genes of hepatitis B virus were introduced into one or both of the nonfusion, LacZ$^+$ transplacement plasmids pBmT524 and

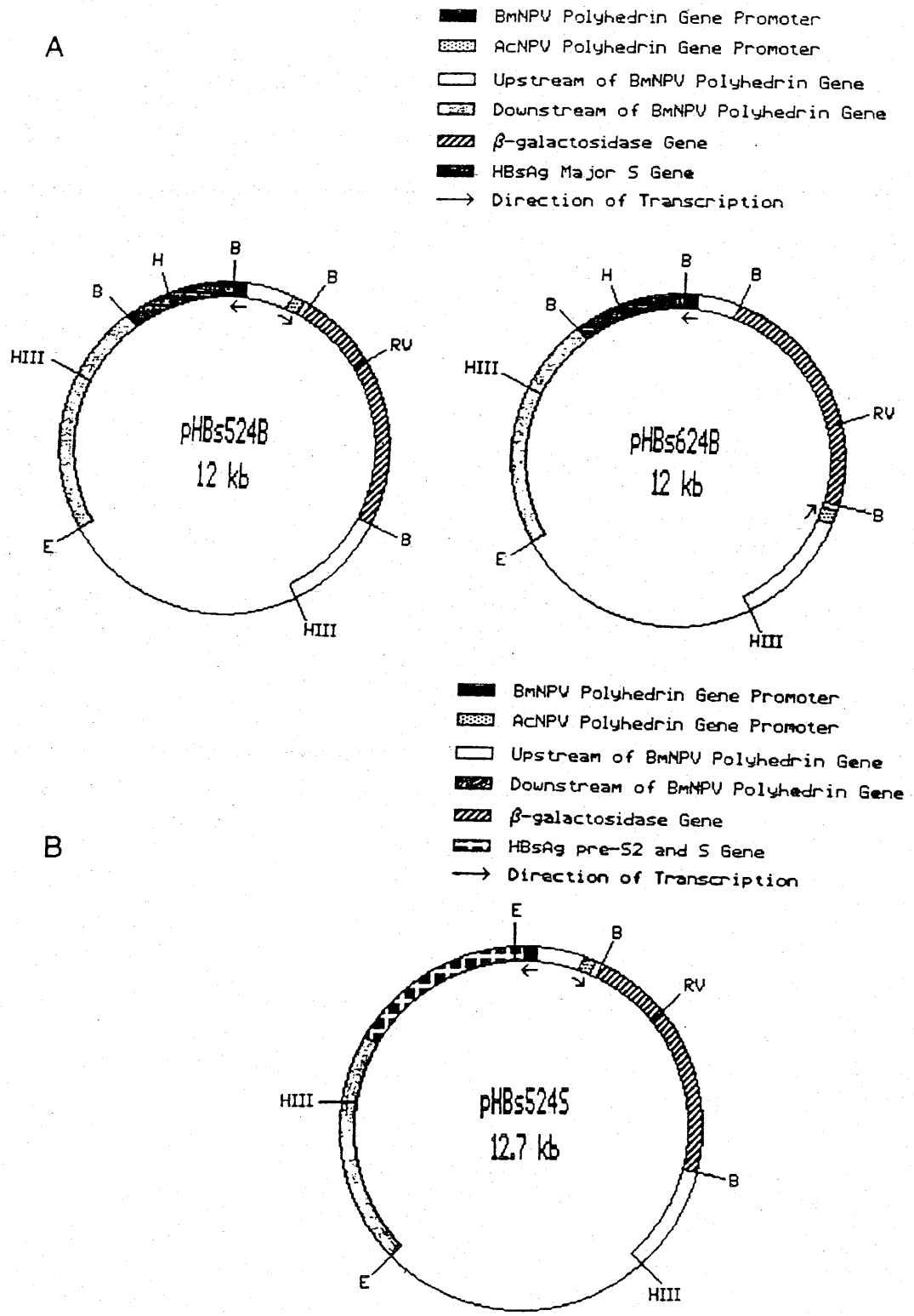


Fig. 5. Structures of (A) pHBs524B, pHBs624B and (B) pHBs524S. Restriction sites are represented as in Fig. 1.

pBmT624, both of which contained a −18 BmNPV polyhedrin promoter (Lin, 1990). Each of the recombinant plasmids, pHBs524B, pHBs624B and pHBs524S (Fig. 5), was cotransfected into BmN cells with BmNPV DNA. Recombinant viruses would be generated from the transfected cells as a result of a DNA exchange between the wild type polyhedrin gene and the flanking sequences of the polyhedrin gene present in the recombinant plasmid. Since both the *lacZ* gene under the control of AcNPV promoter and a HBsAg gene downstream of the BmNPV promoter were flanked by the sequences upstream and downstream of the polyhedrin gene, the recombinant viruses produced from the recombination event contained both foreign genes. Replacement of the wild type polyhedrin gene with the *lacZ* gene produced recombinants which were not only Occ^- but also $LacZ^+$. Through four or five rounds of blue plaque picking, purified recombinant viruses without any polyhedra were obtained.

Production of HBsAg in BmN cells

The presence of HBsAg gene under the control of BmNPV polyhedrin gene promoter in the recombinant virus genome allowed the virus to produce HBsAg late in the infection of the BmN cells. BmN cells infected by purified recombinant virus BmHBs524B, BmHBs624B or BmHBs524S at MOI (multiplicity of infection) of 0.1 were examined for the production of HBsAg on various days post-infection. The amounts of HBsAg were determined for both culture medium and sonicated cell extract using enzyme-linked immunoassay. For comparison, the recombinant virus BmHBs324P, which contained the same construction of HBsAg as those in BmHBs524B and BmHBs625B but without a second polyhedrin gene promoter for the expression

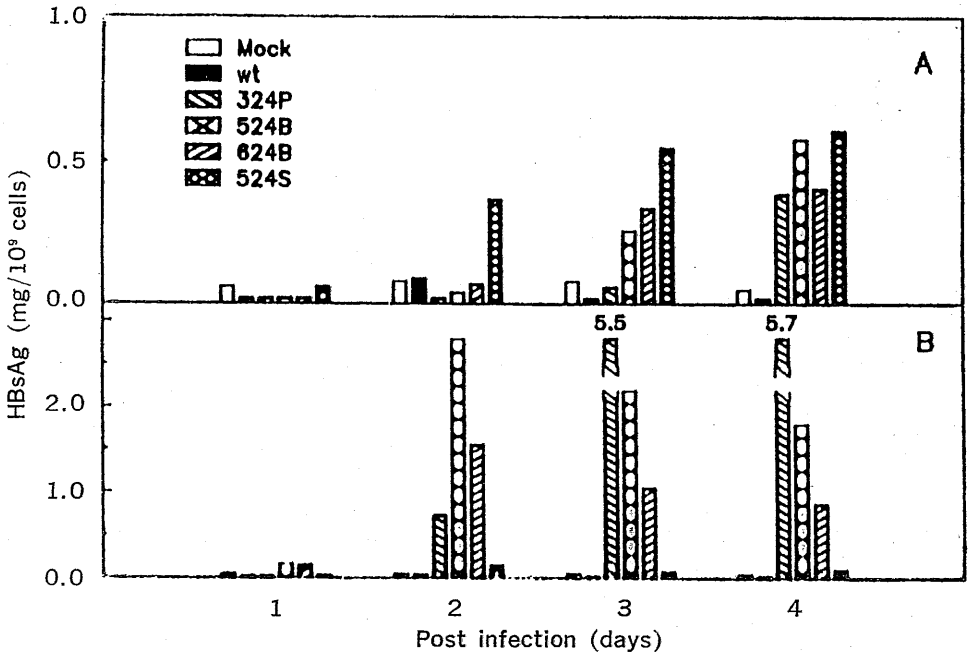


Fig. 6. Comparison of HBsAg expression in BmN cells infected by various recombinant viruses. Mock, infected by media; wt, infected by wild type BmNPV; 324P, infected by BmHBs324P; 524B, infected by BmHBs524B; 624B, infected by BmHBs624B and 624S, infected by BmHBs624S. Both media (A) and cellular extracts (B) were quantitated for HBsAg. The amounts of HBsAg were represented as mg per 10^9 cells.

of the *lacZ* gene, was included in the same experiment. Mock and wild type BmNPV infected cells were also included as negative controls. As shown in Fig. 6, HBsAg detected in the culture medium rose with days after infection for all four recombinants. On the other hand, highest levels of HBsAg in sonicated cell extracts appeared on different days for different recombinants, the second day for BmHBs524B and BmHBs624B and the fourth day for BmHBs324P. In contrast to the above three recombinants, very low levels of HBsAg were detected in the cellular fraction of the virus BmHBs524S, which contained the pre-S2 region of HBsAg gene. Starting from the second day, HBsAg began to be present in the extracellular fraction. The highest total levels of synthesis of HBsAg in the four recombinants BmHB324P, BmHB524B, BmHB624B and BmHB524S were 6.1, 2.8, 1.6 and 0.7 mg/10^9 cells, respectively.

DISCUSSION

An AcNPV polyhedrin/β-galactosidase fusion protein was shown to be synthesized in insect cells at a high level (Pennock *et al.*, 1984). The synthesis of β-galactosidase was temporarily regulated as that of the original polyhedrin protein. Despite the difference in the host range of AcNPV and BmNPV, we demonstrated in this study that an AcNPV polyhedrin/β-galactosidase fusion gene was expressed in silkworm cells. Furthermore, it was also suggested by this study that it is possible to utilize this fusion gene in the screening of recombinant viruses. Comparison of the 5′ flanking region of the polyhedrin gene of AcNPV and that of BmNPV revealed that the two were highly homologous within the 70-bp region upstream of the initiation codon (Rohrmann, 1986). On the other hand, another late gene encoding a 10 kd protein, called p10, appeared to be regulated in the same way as the polyhedrin gene. But its 5′ flanking sequences upstream of the initiation codon were not as conserved (Kuzio *et al.*, 1984). In this study, we chose the AcNPV polyhedrin gene promoter for the expression of the *lacZ* gene and observed that the presence of the AcNPV polyhedrin gene promoter affected the expression of the foreign gene under control of BmNPV polyhedrin gene promoter. Vialard *et al.* (1990) inserted the *lacZ* gene downstream of the p10 gene promoter and detected no difference in the expression of the foreign gene under control of the AcNPV polyhedrin gene promoter. Whether the lower level of synthesis of HBsAg detected in BmHBs524B- and BmHBs624B-infected cells as compared to that in BmHBs324P-infected cells was due to a reduced level of transcription awaits further RNA hybridization experiments.

Synthesis of HBsAg in AcNPV-infected *Spodopters frugiperda* cells (*Sf* cells) has been reported by many laboratories (Kang *et al.*, 1987; Price *et al.*, 1988; Takehara *et al.*, 1988; Lanford *et al.*, 1989). On the other hand, expression of the HBsAg gene in BmNPV-infected silkworm cells was first reported by this laboratory (Hung, 1989). The levels of synthesis of the major S protein as non-fusion protein reported in this study were comparable to those synthesized using AcNPV polyhedrin gene promoter. Since the BmNPV polyhedrin gene promoter utilized for the synthesis of HBsAg in this study was deleted up to the 18th basepair upstream of the initiation codon, the potential for raising the level of expression exists. A four-fold increases in the expression of human α-interferon in silkworm cells was observed when the 19-bp region upstream of the initiation codon was reintroduced (Horiuchi *et al.*, 1987).

The observation that major S protein was detected mainly in the cytoplasmic fraction agrees with that of Lanford *et al.* (1989). In contrast, the introduction of the pre-S2 region in the construction gave two different results. Price *et al.* (1988) detected both extracellular and cytoplasmic HBsAg, whereas we observed only extracellular HBsAg protein. The difference may have arisen as a consequence of the two base changes in the vicinity of the initiation codon in the pre-S2 region used in this study. The base changes made the initiation codon of the pre-S2 more efficient in ribosome binding according to Kozak's rule (Kozak, 1986). The original pre-S2 region was included in the construction of Price *et al.* (1988). Their immunoblot analysis showed that the HBsAg in the extracellular fraction were constituted of major and middle S proteins at a ratio of 2-3:1, whereas only major S was detected in the cytoplasm. Since only the ELISA assays were performed in this study, the ratio of the two proteins detected in the extracellular fraction of BmHBs524S-infected cells was not determined. However, as Scully and Kang (1989) reported, modification of the putative ribosome binding site of pre-S2 promoted preferential synthesis of the middle protein in AcNPV-infected *Sf* cells.

Inclusion of *lacZ* gene in the baculovirus transplacement plasmids made the screening of the recombinant viruses much more convenient. Guided by the blue color on the X-gal plate, one can pick the recombinant virus easily for further purification. Examination for the absence of occlusion bodies or plaque hybridization was entirely eliminated from the whole process. Reintroduction of the missing 18-bp region upstream of the initiation codon may compensate for the reduced synthesis due to the presence of a second polyhedrin gene promoter.

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REFERENCES

Corsaro, B. G. and M. J. Fraser (1988) Transfection of lepidopteran insect cells with baculovirus DNA. *J. Tissue Cult. Methods* **12**: 7-11.

Felgener, P. L., T. R. Gadek, M. Holm, R. Roman, H. W. Chan, M. Wenz, J. P. Northrop, G. M. Ringold and M. Danielsen (1987) Lipofection: a highly efficient, lipid-mediated DNA-transfection procedure. *Proc. Natl. Acad. Sci. USA* **84**: 7413-7417.

Horiuchi, T., Y. Marumoto, Y. Saeki, Y. Sato, M. Furusawa, A. Kondo and S. Maeda (1987) High-level expression of the human α-interferon gene through the use of an improved baculovirus vector in the silkworm, *Bombyx mori*. *Agric. Biol. Chem.* **51**: 1573-1580.

Hung, H. M. (1989) Construction of transplacement plasmids using *Bombyx mori* nuclear polyhedrosis virus. Master's Thesis, National Chung Hsing University.

Kang, C. Y., D. H. L. Bishop, J. S. Seo, Y. Matsuura and M. Choe (1987) Secretion of particles of hepatitis B surface antigen from insect cells using a baculovirus vector. *J. Gen. Virol.* **68**: 2607-2613.

Kozak, M. (1986) Point mutations define a sequence flanking the AUG initiator codon that modulates translation by eucaryotic ribosomes. *Cell* **44**: 283-292.

Kuzio, J., D. Z. Rohel, C. J. Curry, A. Krebs, F. B. Carstens and P. Faulkner (1984) Nucleotide sequence of the p10 polypeptide gene of *Autographa californica* nuclear polyhedrosis virus. *Virology* **139**: 414-418.

Landford, R. E., V. Luckow, R. C. Kennedy, G. R. Dreesman, L. Notvall and M. D. Summers (1989) Expression and characterization of hepatitis B virus surface antigen polypeptides in insects cells with a baculovirus expression system. *J. Virol.* **63**: 1549-1557.

Lin, P. R. (1990) Beta-galactosidase gene of *Escherichia coli* as a selection marker for expression vector of *Bombyx mori* nuclear polyhedrosis virus. Master's Thesis, National Chung Hsing University.

Luckow, V. A. and M. D. Summers (1988) Trends in the development of baculovirus expression vectors. *Biotechnology* **6**: 47-55.

Maeda, S. (1989a) Expression of foreign genes in insects using baculovirus vectors. *Ann. Rev. Entomol.* **34**: 351-372.

Maeda, S. (1989b) Gene transfer vectors of a baculavirus, *Bombyx mori* nuclear polyhedrosis virus, and their use for expression of foreign genes in insect cells. In Invertebrate Cell System and Applications, ed. J. Mitsuhashi. Boca Raton, Fla. CRC.

Maniatis, T., E. F. Fritsch and J. Sambrook (1982) Molecular cloning. A laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.

Miller, L. K. (1988) Baculovirus as gene expression vectors. *Ann. Rev. Microbiol.* **42**: 177-199.

Pennock, G. D., C. Shoemaker and L. K. Miller (1984) Strong and regulated expression of *Escherichia coli* β-galactosidase in insect cells with a baculovirus vector. *Mol. Cell. Biol.* **4**: 399-406.

Price, P. M., A. Mohamad, A. Zelent, A. R. Neurath and G. Acs (1988) Translational selection in the expression of the hepatitis B virus envelope proteins. *DNA* **7**: 417-422.

Rohrmann, G. F. (1986) Polyhedrin structure. *J. Gen. Virol.* **67**: 1499-1513.

Scully, L. J. and C. Y. Kang (1989) Preferential synthesis of the middle protein of hepatitis B surface antigen by modification of the putative ribosome binding site flanking the pre-S2 translation initiation codon. Abstracts of Cold Spring Harbor Meeting on Hepatitis B Virus, pp. 135. Cold Spring Harbor Laboratory, Cold Spring Harbor, N. Y.

Summers, M. D. and G. E. Smith (1987) A manual of methods for baculovirus vectors and insect cell culture procedures. *Tex. Agric. Exp. Stn. Bull.* No. 1555.

Takehara, K., D. Ireland and D. H. L. Bishop (1988) Co-expression of the hepatitis B surface and core antigens using baculovirus multiple expression vectors. *J. Gen. Virol.* **69**: 2763-2777.

Vialard, J., M. Lalumiere, T. Vernet, D. Briedis, G. Alkhatib, D. Henning, D. Levin and C. Richardson (1990) Synthesis of the membrane fusion and hemagglutinin proteins of measles virus, using a novel baculovirus vector containing the β-galactosidase gene. *J. Virol.* **64**: 37-50.

半乳糖苷酶基因供作家蠶核多角體病毒表現載體之選標

胡念台　林佩如　侯豐男

利用家蠶核多角蛋白基因啓動子的置換載體已於本實驗室中構築成功，並經殖入B型肝炎表面抗原基因，成功地於家蠶細胞及蠶體中合成蛋白質。篩選重組病毒時，原本作爲篩選標記的核多角體之形成與否往往不易判斷，只得仰賴核酸雜交法，既費時又不精確。爲增進置換載體之可用性，本研究採用大腸菌之半乳糖苷酶基因，完成一組具兩個核多角蛋白基因啓動子之置換載體，利用其中一個啓動子合成半乳糖苷酶提供篩選標記，利用另一啓動子合成異源蛋白。經殖入B型肝炎表面抗原基因，證實以半乳糖苷酶基因作爲篩選標記，既便利又省時，經四至五次單一溶斑挑選，即可選到不雜有任何核多角體之純系重組病毒。